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TRP Channels and Pain

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Abstract

Since the molecular identification of the capsaicin receptor, now known as TRPV1, transient receptor potential (TRP) channels have occupied an important place in the understanding of sensory nerve function in the context of pain. Several TRP channels exhibit sensitivity to substances previously known to cause pain or pain-like sensations; these include cinnamaldehyde, menthol, gingerol, and icillin. Many TRP channels also exhibit significant sensitivity to increases or decreases in temperature. Some TRP channels are sensitized *in vitro* by the activation of other receptors such that these channels may be activated by processes, such as inflammation that result in pain. TRP channels are suggested to be involved in processes as diverse as sensory neuron activation events, neurotransmitter release and action in the spinal cord, and release of inflammatory mediators. These functions strongly suggest that specific and selective inhibition of TRP channel activity will be of use in alleviating pain.

INTRODUCTION

In the past few decades a tremendous amount of research has been performed in an attempt to elucidate and understand the factors and mechanisms involved in normal sensory and pain perception. Preclinical research has uncovered a multitude of factors, pathways and mechanisms that are involved in the development and maintenance of pathological pain-like sensory sensitivity in animal models. Key discoveries in the pain field include the description of injury-induced alteration of neuronal function [1], the discovery of changes in peripheral neuron functioning and in neuronal processing within the spinal cord [2, 3], the development of animal models of neuropathic pain [4-7], and the role of immune and glial cells in the development of pain [8-10]. These discoveries have generated optimism that new therapeutic regimens may be forthcoming to alleviate abnormally heightened and spontaneous pain.

However, despite these advances the translation of this preclinical research to the treatment of pain has been slow to occur. This is partly due to the lack of understanding of the clinical role of molecular and physiological mechanisms that are key to animal research models. This is evident by the relative lack of mechanistically novel therapeutic agents that have entered into the clinic for the treatment of pain in recent years. Although the use of new agents for pain has risen (for example, gabapentin and the COX-2 inhibitors), the mainstay of therapy remains agents such as opiates and non-steroidal anti-inflammatory drugs (NSAID) that have been around for a number of years, even centuries. Of the newer agents, the COX-2 inhibitors provided an extremely promising

line of therapy for inflammatory conditions before side-effects have cast a shadow over the use of the class as a whole. Neurontin (gabapentin) has offered insight into a potentially new mechanism, the $\alpha 2\delta$ calcium channel [11]. Furthermore, although Neurontin is one of the most prescribed pain relief medications for neuropathic conditions, there is still room for improvement. In clinical trials of post-herpetic and trigeminal neuralgia the number-needed-to-treat (NNT) was approximately 3-4 [12]. A more potent successor to gabapentin, pregabalin (LYRICA), has recently reached the market. It appears to improve on the potency of gabapentin and may provide further clinical validation for the $\alpha 2\delta$ mechanism. Finally, ziconotide (PRIALT), a conopeptide N-type calcium channel blocker also has beneficial use in treating pain, yet side effects and its route of administration (intrathecal delivery) limit its use to opiate-refractory, severe chronic pain. In summary, clinical therapy for pain relies on agents that do not provide adequate relief in a large number of patients to which they are prescribed and, therefore, can be significantly improved upon [13]. There remains a great need for new therapeutic agents acting via novel mechanisms in this field of medicine.

A recent development in the search for novel analgesics is the advance into clinical testing of small molecule antagonists of the TRPV1 channel [14]. TRPV1 is a member of the TRP ion channel family and is the receptor for capsaicin [15]. TRPV1 has been shown to be an extremely promising target for the development of novel analgesic therapeutics and beckons examination of further relevant members of this class of channels. Indeed, in the years that have followed the isolation and cloning of TRPV1, additional TRP family members have been identified (Figure 1), which are expressed in

sensory fibers, and which are implicated in sensory processing, including TRPV2, TRPV3, TRPV4, TRPA1 and TRPM8 [16]. These and other TRP channel members may hold promise as relevant targets for the discovery of the next generation of analgesic agents.

Allogenic natural substances and TRP channels

Perhaps the most exciting insight into the molecular nature of pain in the last 10 years has been the identification of proteins that serve as functional receptors for chemicals known to cause pain. In fact, these algogenic molecules have provided tremendous tools with which to identify and characterize these receptors (reviewed by [17]). The first to be identified was TRPV1 as the receptor for capsaicin and related vanilloids [15]. TRPV1 is also the receptor for other algogenic substances or irritants, including resiniferatoxin, a vanilloid [15, 18], piperine, the primary irritant in black pepper [19], gingerol [20], and eugenol, a component of clove oil [21]. Venoms from cnidarians, such as jellyfish, and the spiders *Psalmopoeus cambridgei* and *Ornithoctonus huwena* are also activators of TRPV1 [22, 23].

The TRPA1 channel is also activated by gingerol and eugenol, but more importantly is the receptor for mustard oil [24, 25]. The effects of mustard oil on TRPA1 appear to be mediated largely through allyl isothiocyanates which activate the channel via reversible, covalent modification of cysteine residues [26, 27]. Cinnamaldehyde, which is found in cinnamon oil and is the most specific TRPA1 ligand [24], also activates TRPA1 via covalent modification [27]. TRPA1 is also activated by extracts of other isothiocyanate-

containing compounds, including horseradish, and garlic, which contains a thioosulfinate [24, 25, 28, 29]. It is also activated by wintergreen oil [24]. Curiously, delta(9)-tetrahydrocannabinol (THC), a psychoactive ingredient in marijuana, which is known to mediate analgesia, is also a reported activator of TRPA1 [25].

Several TRP channels are also activated by natural substances which are associated with certain sensory and/or medicinal effects, but not necessarily pain. TRPM8 has been termed the ‘minty-cool’ receptor [30], a reflection of its role as a receptor for menthol and eucalyptol and other selected monoterpenes [31-33]. TRPV3 is also activated by natural products, including carvacrol (from oregano), thymol (from thyme), eugenol, and camphor [34, 35]. Interestingly, vanillin, the primary flavoring component of vanilla bean extract, and ethyl-vanillin appear to be the most effective of the TRPV3 natural product activators described to date [34]. Finally, a compound called bisandrographolide A, which is found in extracts of the Chinese medicinal plant *Andrographis paniculata*, is an activator of TRPV4 [36].

Many of these ligands exhibit promiscuous binding to TRP channels. For example, eugenol activates TRPV1, TRPA1, TRPM8 and TRPV3 [21, 24, 34], and carvacrol activates TRPV3 and TRPA1 [34]. In contrast, MacPherson and coworkers [37] have shown that menthol activates TRPM8 and TRPV3, but inhibits TRPA1. Conversely, cinnamaldehyde activates TRPA1, but high concentrations inhibit TRPM8 [37]. Capsaicin, it would seem, is the only selective ligand of the lot. Thus, while it is clear that TRP channels are important mediators of natural products’ effects, our understanding

of the contribution of specific channels to a particular compound's pharmacological effects is still emerging.

TRP Channels as coincident sensors of temperature and phospholipid metabolism:

Implications for nociceptive processing

Certain TRP channels, particular channels expressed in skin and sensory neurons (DRG), exhibit exquisite sensitivity to changes in temperature. Indeed, the thermal sensitivities of TRPV2 and TRPV3 were a defining feature of their activation when first molecularly characterized. Of particular interest has been the finding that each thermally sensitive TRP channel (thermo TRP) exhibits a unique activation threshold *in vitro*; for example, TRPV1 is activated at temperatures of ~43 °C, TRPA1 at ~17 °C, and TRPV4 at 27 °C, TRPV3 at 33 °C, TRPM8 at 26 °C, and TRPV2 at >50 °C (reviewed in [38-40]). Since these “thermoTRPs” exhibit unique, albeit overlapping distributions within sensory nerve fibers, it is plausible that thermoTRPs mediate unique cellular responses of sensory neurons to temperature change. In fact, genetic deletion of TRPV1 [41, 42], TRPV3 [35], or, in some studies, TRPA1 [44] results in alterations in sensitivity to warm/hot (TRPV1, TRPV3) or cold temperatures (TRPA1). In addition to their function as peripheral temperature sensors, an emerging literature indicates that TRP channels participate in aspects of thermoregulation [45].

The ability to detect noxious cold or hot temperatures, particularly at noxious temperatures (below 17 °C or above 43 °C), has suggested a role for channels such as TRPA1, TRPV1, and TRPV2 in sensing painful temperature conditions, roles that to this

point have been corroborated by additional studies (described in detail below).

Interestingly, TRPV3 $-/-$ mice do not develop thermal hyperalgesia in response to inflammation [35], even though TRPV3 exhibits increased current responses to repeated heat stimulation [45]. Thus, thermal sensitivity *per se* is not sufficient to drive nociception.

A number of TRP channels have been shown to be activated by phospholipid signaling molecules such as diacylglycerol (DAG). Activation of phospholipid metabolism is an important downstream event activated by G protein-coupled receptors linked to nociceptive signaling, such as bradykinin and prostaglandins. TRPA1, TRPV3, and TRPV4 are all activated by increased phospholipase C (PLC) activity [24, 34, 46], and TRPV1, which is negatively regulated by phosphatidylinositol bis-phosphate (PIP2), is sensitized by increased PLC activity [47]. TRPV4 and TRPM8 are activated by the products of phospholipase A2 activity [48, 49], whereas PLC activation desensitizes TRPM8 activity [50]. In some cases, lipid metabolites are direct activators of these channels, as exemplified by the activation of TRPV1 by anandamide, 12-hydroperoxyeicosatetraenoic acid (12-HEPTE), and N-acyl taurines [51, 52] and the activation of TRPM8 by lysophospholipids [49].

Perhaps the most intriguing aspect of heat and phospholipid sensitivity of TRP channels relates to their ability to integrate multiple stimuli. Tominaga and coworkers [53] provided the first evidence of integration with the demonstration that the temperature activation threshold for TRPV1 could be shifted to cooler temperatures by low pH

solutions (pH 6.4 vs. pH 7.4). This was followed by the demonstration that increased phosphorylation of TRPV1 via protein kinase C could also sensitize the channel to heat [54, 55]. Finally, mildly low pH (6.8) in combination with a moderate increase in PKC activity can result in activation of TRPV1 current responses at 37 °C [56], thus, demonstrating the ability of TRPV1 to activate a response under physiological conditions (tissue acidification, release of pro-inflammatory mediators, increased local blood flow and temperature) that might be found during inflammatory pain processes. Other data supports multimodal integration roles for TRPA1 [57], TRPV4 [46, 58], and TRPM8 [49].

In vivo evidence for the importance of TRP channels in pain

In view of the emerging molecular pharmacology of TRP channels expressed in sensory neurons, particularly those channels which mediate the effects of well known algogenic substances, it is not surprising that significant attention has been focused on the role these channels in pain. Numerous small molecule TRPV1 antagonists have been reported which further support its central role in pain. Genetic knockdown and knockout strategies have been deployed to study TRPV1, TRPA1, TRPV4, and TRPM8.

TRPV1

TRPV1 has been the subject of intense study and academic review [14, 18, 51, 59-61]. In brief, TRPV1 is highly expressed in a subset of sensory neurons fibers generally referred to as nociceptors. This site of expression is consistent with the ability of TRPV1 agonists, like capsaicin, to trigger nociception in animals. Activation of TRPV1 in

sensory fibers also releases neuropeptides such as substance P and calcitonin gene-related peptide causing increased blood flow and edema, sometimes referred to as neurogenic inflammation [18]. Perhaps the most outstanding feature of TRPV1 is, as noted above, its ability to integrate the effects of multiple noxious stimuli and inflammatory mediators, a characteristic which combined with TRPV1's cellular localization provides an intriguing rationale for the utility of TRPV1 antagonists in the treatment of pain.

In fact, numerous TRPV1 antagonists have been reported which exhibit efficacy in a wide range of pre-clinical pain models [14, 51]. These reports corroborate to a significant extent the phenotypes of TRPV1 $-/-$ mice in experimental pain, and are in agreement with the effects of TRPV1 knockdown via anti-sense oligonucleotide or RNA interference [62, 63]. Several TRPV1 antagonists are currently in Phase II clinical trials for pain (SB-405798 and MK2295/NGD 8243) so a first look at TRPV1 as a mechanistic target for pain should be forthcoming shortly.

TRPA1

TRPA1 is expressed mostly in subsets of small DRG neurons and has been considered to be a noxious cold-sensing channel as it is activated by temperatures lower than 17°C, lower than that of TRPM8, the “minty-cool” or menthol receptor [30, 40]. It is also activated by bradykinin in a bradykinin receptor-dependent manner and may potentially function as a signal integrator much like TRPV1 [24]. TRPA1 expression is not limited to DRG neurons, as it is expressed on hair cell stereocilia, thus, implying a role in

hearing. In fact, antisense experiments provided support for a role of TRPA1 in hearing [64], but TRPA1 knockout mice do not display deficits in auditory responses [43, 44].

Antisense and knockout studies have identified a role for TRPA1 in pain mechanisms. TRPA1 expression is induced following both inflammatory and nerve injuries and antisense knockdown of the channel resulted in decreased cold hyperalgesia with little effect on thermal (heat) hyperalgesia or mechanical allodynia resulting from these conditions [65]. More recent genetic TRPA1 knockout studies have identified a loss of mustard oil- and bradykinin-induced hypersensitivity in knockout animals with no effect seen on CFA-induced thermal hypersensitivity or spared nerve injury-induced mechanical allodynia [43, 44]. Interestingly, although data has implicated TRPA1 in cold sensing, data from knockout mice in this regard is mixed and further studies have failed to correlate this channel with cold sensing neurons [25]. Therefore, the role of TRPA1 in normal cold sensing remains controversial and further research is needed to elucidate its physiological function in temperature sensing. However, reports have suggested that post-injury, TRPA1 plays a role in cold hypersensitivity [65, 66] indicating that TRPA1 may be a potentially attractive target for pathophysiological cold pain.

TRPM8

TRPM8 is a temperature and ligand gated channel that is activated by temperatures in a modestly cool range, activating at approximately 26 °C and saturating at approximately 8 °C. This activation range overlaps with TRPA1, but extends to warmer temperatures [31].

Despite the cool temperature sensitivity of TRPM8, antisense oligonucleotides directed against TRPM8 conferred no alteration in nerve injury-induced cold hypersensitivity [66]. On the other hand, activation of TRPM8 has been demonstrated to produce analgesia, an effect that is lost with TRPM8 antisense oligonucleotide treatment [67]. These experiments suggest a role for TRPM8 as a cold analgesia transduction mechanism therefore accounting for the cooling analgesia for which menthol has long been employed.

TRPV4

TRPV4 is a channel that can be heat and ligand-activated similar to other TRP channels. Ligands that can activate this channel include phorbol derivatives [68] and endocannabinoid/arachidonic acid metabolites (5',6'-EET; [48]). Temperature activation is in a warm range (greater than 27 °C) lower than that required to activate TRPV1 [69, 70]. Interestingly, it can also be activated by osmolarity and low pH and may serve as an integrator between mechanosensation and osmoregulation [71, 72]. Consistent with a role in osmoregulation, TRPV4 is expressed in the kidney and in circumventricular organs in the CNS [72-74]. TRPV4 is also expressed in dorsal root ganglia, trigeminal ganglia, and mechanosensory terminals in the skin as well as cochlear hair cells and vibrissal Merkel cells indicating a potential role in mechanosensation. Indeed, studies have identified a function for TRPV4 in normal hearing [71, 74-76].

TRPV4 knockout studies have demonstrated a role for TRPV4 in acid and mechanical sensing whilst these animals displayed normal heat and touch sensation [77, 78]. Further

studies have demonstrated a role for TRPV4 in thermal sensing under inflammatory conditions [79] and in environmental temperature sensation [80] consistent with channel activation by heat. Therefore, although its role in normal, uninjured physiology is unclear, TRPV4 appears to be an important player in pathological sensory perception. A further intriguing finding indicates a potentially key role of TRPV4 in chemotherapy-induced neuropathy. In a study by Alessandri-Haber and colleagues [81], antisense treatment targeting TRPV4 completely reversed Taxol-induced mechanical hypersensitivity. As chemotherapy-induced neuropathy is a common, potentially irreversible side effect caused by some chemotherapeutic agents [82], this data suggests a potential mechanism to combat this side effect. Further studies with both knockout animals and antisense administration have demonstrated a role for TRPV4 in inflammatory pain, but not in normal pain sensing [58]. However, a potential role in central osmoregulation may limit the therapeutic benefit of targeting TRPV4 [83].

Emerging functions of TRP channels and their implications for pain

From the perspective of pain research, much of the focus in the TRP channel field has been on the functional activity of channels expressed in sensory neurons.

Mechanistically, this focus has largely been on TRP channels as temperature sensors and mediators of natural substance effects. However, other TRP channel functions have been described, including the early description of TRP channels as mediators of calcium release-activated currents (see below). In addition, TRP channels are found in cells of the central nervous system and in diverse, non-neuronal cell types all of which participate in generation, sensing, and processing of pain signals (Figure 2).

TRP Channels and Mechanical Pain

One of the unanswered questions involved in pain research concerns the mechanisms underlying mechanical sensitivity and hypersensitivity. Although a number of channels have been implicated in playing a role in mechanical sensing, there has been very little definitive evidence for a universal mechanical transduction mechanism. Members of the TRP channel family represent reasonable candidates as peripheral sensory mechanotransducers [84]. Evidence for mechanosensory properties of TRP channels ranges from drosophila to humans channel studies and from research suggesting a role in hearing in many species (described above). For example, two TRPV-related proteins, OSM-9 and OCR-2, have been shown to play mechanotransduction roles in *C. elegans* [85, 86]. Furthermore, homologs of the drosophila TRPN family member *nompC* have been found to play a role in the drosophila mechanotransduction and zebrafish hearing [87, 88], although no human homolog for this channel is known to exist [89]. Finally, the drosophila channel *Painless*, a TRPA family member, appears to be important in certain aspects of mechanotransduction associated with touch [90]. Collectively these studies suggest that the TRP channel superfamily plays a role in mechanotransduction and, potentially mechanical nociception or hypersensitivity in pain-like states.

Yet, evidence to date from mammalian studies is far from complete. Although evidence from some studies suggests a role of TRPA1 in mechanical nociception, other reports indicate that this role is not clear and requires further elucidation [43, 44, 65]. A compelling case can be made for a role of TRPV4 in mechanical sensing. Studies have

observed a mechanical sensory deficit in both knockout and antisense studies [78, 81]. Interestingly, both TRPA1 and TRPV4 have been implicated as force transducers in auditory hair cells [64, 76] although their role in hearing is debated [91]. Despite the lack of clarity regarding the role of a number of TRP channels in mechanical sensory transduction, the data could be indicative of a remarkable diversity and redundancy in such systems thus allowing a fault in one mechanosensory mechanism to be compensated by another. This would certainly allow for subtle or limited effects of genetic modifications in knockout or transgenic studies. Furthermore, as signal integrators, certain physiological stimuli can affect the activation of a channel to other stimuli. For example, temperature can affect the chemical, osmotic and mechanical (shear stress) responsiveness of TRPV4 (the channel is more responsive to these stimuli at 37°C than at room temperature) and the ligand gating of TRPA1 [57, 92].

In the context of mechanical sensitivity, TRPV1 appears to be something of an enigma. No study to date has demonstrated direct mechanical activation of TRPV1. Nonetheless, intriguing anatomical and physiological evidence implies that TRPV1 may be involved in mechanically-induced response. Meissner corpuscles, which are suggested to mediate low threshold mechanosensitive responses, contain a subset of sensory fibers that exhibit TRPV1 immunoreactivity. This observation provides circumstantial evidence that TRPV1-expressing fibers might be involved in mechanical hypersensitivity [93]. TRPV1 antagonists and TRPV1 antisense oligonucleotide exhibit efficacy in inflammation and nerve injury-induced mechanical hypersensitivity [14, 51, 63]. Additionally, TRPV1 knockout mice exhibit decreased visceral fiber responses to stretch of the bladder [94],

jejunum [95], and colon [96]. Despite this supporting evidence, TRPV1 $-/-$ mice do not exhibit any alteration in mechanical hypersensitivity in pain models [15, 42].

(REFERENCE Bolcseki, 2005????)

Roles for TRP channels in peripheral and spinal processing of nociception

Activation of nociceptive pathways can be modified on an activity-dependent basis, which is manifest as a progressive increase in response due to repetitive stimuli. This is typically termed activity-dependent plasticity or sensitization [97]. Generally there are two types of such sensitization that are relevant to pain systems, namely, autosenitization of nociceptor nerve terminals, and sensitization of dorsal horn neurons and their signaling, often termed “windup”. Both types of sensitization are relevant for members of the extended TRP family.

Nociceptor autosenitization has been amply demonstrated with TRPV1 being at the center due to its integrative properties as described above. TRPV1 also plays a key role in the depolarization of nociceptors. The activation of TRPV1 channels results in localized sodium and calcium ion influx, causing non-propagated neuronal generator potentials [98]. When such generator potentials are sufficiently strong, neuronal depolarization occurs. This molecular integration coupled with channel sensitization per se (ie, by phosphorylation as described above) would appear to augment its ability to be intimately associated with and responsible for nociceptive neuronal transmission in the hyperalgesic state.

Mustard oil has long been used as a selective activator of C-fibers and sensitizer of dorsal horn neurons [99], and is a selective activator of TRPA1 channels as discussed above. It is now appreciated that TRPA1 is coexpressed within a subset of TRPV1 expressing sensory neurons [100], and as such it appears that TRPA1 channels play a key role in central sensitization regarding both thermal and mechanical hypersensitivities. Dorsal horn lamina 1 noci-responsive neurons and wide dynamic range neurons contain neurokinin-1 receptors, and mustard oil applied to the relevant receptive field has been shown to profoundly sensitize the responses of these neurons to thermal stimulation [101].

Meanwhile, mustard oil does not sensitize the responses of these lamina 1 neurons to noxious mechanical stimulation, demonstrating specificity to the sensitizing effects of mustard oil in this spinal cord region [101, 102]. On the other hand, spinal cord lamina V neurons play important roles in mechanical and thermal sensitivity, and receptive field application of MO profoundly sensitizes lamina V neurons to mechanical stimulation in mice [101, 103]. A role for TRPA1 in triggering central sensitization is strongly suggested by these studies. However, TRPA1 knockouts develop a mechanical hypersensitivity in response to peripheral nerve injury that is similar to wild-types [44]. Therefore, mechanical hypersensitivity induced by peripheral nerve injury and by mustard oil application may not be equivalent. The role of TRPA1 in sensitization requires confirmation using TRPA1 antagonists to understand its contribution to hypersensitivity in pain models.

Another relevant aspect of nociceptive processing relates to the profound expression of TRPV1 [53], TRPM8 [104], and potentially TRPA1 (suggested by its strong DRG expression), in the sensory neuron projections to the spinal dorsal horn (Figure 2). This presynaptic localization suggests the potential for these TRP channels to be involved in the synaptic release of neurotransmitters. Indeed, capsaicin is well known to trigger release of neurotransmitter when directly applied to the spinal cord [105]. And, menthol increases synaptic transmission in a DRG-dorsal horn co-culture system [104]. In spite of these data, very little is known about the function of these channels in pre-synaptic physiology. Recently, the TRP channel TRPM7 has been shown to effect acetylcholine release from sympathetic neurons suggesting that TRP channels can play a role in physiological synaptic function [106]. Moreover, there is evidence that central nervous system sites are important in nociceptive processing. A-795614, a TRPV1 antagonist that does not penetrate the CNS well, exhibits limited efficacy in models of pain that exhibit a strong component of central sensitization. In contrast, A-784168 and other TRPV1 antagonists reported in the literature with good CNS exposure, give good efficacy in those models [107].

With data at hand, one can conclude that TRPV1 and TRPA1 play important roles in sensitization as related to mechanical and thermal pain. These and other TRP channels may also be involved in the regulation of neurotransmitter release from spinal efferents to spinal dorsal horn neurons (Figure 2). It will be important to understand the extent to which these and other TRP channel members affect nociceptive processing, particular given the importance of central sensitization in chronic pain.

Potential role for TRP channels in inflammation: Implications for pain

Research on TRP channels and pain has largely been focused on aspects of nociceptive processing. However, sensory neurons are also implicated in neurogenic inflammation, a process in which neuropeptides, such as substance P and CGRP, are released from the peripheral termini of sensory fibers resulting in peripheral vasodilation, vascular leak, and effects on immune cells. These responses are thought to contribute to certain disorders including migraine, in which CGRP released from trigeminal ganglion peripheral nerve endings mediates a vasodilatory, pro-algesic effect [108]. Since capsaicin has been widely used to trigger experimental neurogenic inflammation, TRPV1 is implicated as an important player in that process [18]. Indeed, capsazepine significantly inhibits the development of inflammation in an enteritis model [109].

The effect of sensory fiber activation on inflammation, however, is a two-way street. Inflammatory processes generate molecules, such as the pro-inflammatory cytokine, TNF α , that are released from activated macrophages, mast cells, and other immune cells, and which have direct and indirect effects on nociceptors [110]. An important characteristic of some immune cell types, including T lymphocytes and mast cells, is the presence of receptor activated calcium mobilization. This can be mediated by inositol-3-phosphate (IP3)-activated receptors and non-IP3-activated receptors as well as store-operated calcium currents (SOC) or calcium release-activated calcium current (CRAC). These currents are thought to play a pivotal role in the functional outcome of cell activation, such as degranulation, reactive oxygen generation, or proliferation [111]. It

has been appreciated for some time that TRP channels, particularly TRPC subfamily members, are sensitive to the intracellular signaling molecule diacylglycerol [112], placing TRP channels in the pathway via which activation of phospholipase (by, for example, G protein coupled receptor engagement) can mediate calcium mobilization and/or influx. Numerous investigations have also suggested a connection between SOC and CRAC currents and TRP channels [113]. Recent data suggests that other proteins (STIM1 and CRACM1) may be necessary and sufficient to mediate CRAC [114], but TRP channels may interact with these proteins in some cases to mediate SOC currents [115].

One example of the connection between TRP channels and receptor-activated currents is TRPC3, which is activated by B cell receptor in an avian B cell line [116], and T cell receptor in human Jurkat T cells [117]. Another example is TRPV6, which exhibits properties of calcium release-activated current in Jurkat T cells [118]. Since receptor-activated calcium currents are important for T cell activation [119], it is tempting to speculate that TRPC3, TRPV6, and other TRP channels are involved in T cell events that might result in the release of factors, such as TNF α , that mediate pain.

TRPV2 is the target of protein kinase A phosphorylation-dependent mast cell surface expression, and contributes to functional mast cell degranulation [120]. However, TRPV1 is also expressed in mast cells [121], raising the possibility that, like in sensory neurons, TRPV1 sensitization may mediate activation of these cells. The release of

histamine, serotonin, and proteases from mast cells may contribute to hyperalgesia via increased local blood flow or edema and through direct actions on nociceptors.

Two members of the TRPM family have potential roles in immune cell activation.

TRPM2 is expressed in granulocytes [122] and lymphocytes [123], among other cell types. It is activated by NAD such that increased H_2O_2 levels during oxidative burst in granulocytes mediates calcium influx via NAD activation of TRPM2 [122, 124].

Adenosine diphosphoribose (ADPR) is also an activator of TRPM2. Increased intracellular ADPR levels observed in Jurkat T cells after concanavalin A treatment activate TRPM2 [123] suggest a role for this channel in T cell activation. TRPM4 has also been implicated in T cell activation events by regulating intracellular calcium levels. Inhibition of TRPM4 function results in increased phytohemagglutinin (PHA)-induced IL-2 secretion [125], while facilitation of TRPM4 currents decreases PHA IL-2 release [126].

It is clear that many TRP channels are expressed in immunocytes, including lymphocytes, neutrophils, macrophages, mast cells, and glia [127-130], but, with some intriguing exceptions (noted above), the functional roles of TRP channels in immune cells are unclear. In addition, multiple TRP channels can be found functionally expressed in the same cell type; for example, TRPC3, TRPV6, and TRPM2 expression in T cells as described above. The ways in which multiple TRP channels independently or in concert effect immune cell function remain to be elucidated.

Conclusions

In the almost 20 years since their discovery, TRP channels have been implicated in a broad range of physiological activities. The identification of the receptor for capsaicin in 1997 as a TRP channel [15] heralded a new era in the molecular understanding of pain sensation. Today, we appreciate that at least 6 temperature sensitive TRP channels are expressed in sensory neurons, and most of these are also receptors for sensory-active substances such as menthol, mustard oil, and venoms. Pharmacological inhibitors and genetic knockout and knockdown strategies demonstrate that inhibition of these TRP channels may be effective therapeutic strategies for pain. Emerging data implicates TRP channels in sensing mechanical stimuli, in spinal neurotransmitter release, and in immune cell function, all of which underscore the potential importance of TRP channels as targets for the next generation of pain therapies.

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Figures

Figure 1: Timeline of research milestones in study of TRP channels in pain.

TRPV1 has been the most studied of the TRP channels with respect to pain, and, thus, research milestones in the area are biased toward TRPV1 (right side of timeline). Note that 10 years after the description of capsaizepine, Phase 1 trials were initiated with two TRPV1 antagonists. Other research milestones are centered on the discovery of other TRP family channels with sensory transduction capabilities or with functions in immune cells (left side of timeline). The role of TRPM8 in pain is unclear, but its discovery was the first description of a cool/cold temperature sensitive sensory channel.

Figure 2: Cartoon illustration of cell types important in the generation and/or

sensory transduction of pain. Many TRP channels thought to be involved in pain are expressed in dorsal root ganglion neurons that exhibit sensory projections to peripheral target organs (afferents), such as skin and gastrointestinal tract, as well as projections to the spinal cord (efferents). The circle overlay on the spinal column illustrates a cross-section of the spinal cord, wherein sensory efferents project to the dorsal areas. Certain cell types in peripheral tissues may be involved in the transduction of sensory information to sensory nerve fibers, and also may express TRP channels; for example, TRPV3 and TRPV4 are expressed in keratinocytes [15] and TRPV1 is expressed in bladder lumen epithelial cells [135]. In the spinal cord, TRP channels are found in both sensory fiber ends as well as in certain cell bodies. TRP channels are also expressed in a variety of immune cells in which they may participate in the release of mediators that activate nociception. TRP channels are also found in microglia, which are important

cells in the pathogenesis of neuropathic pain [8-10]. However, the functional contribution, if any, of TRP channels to microglia function is unknown.

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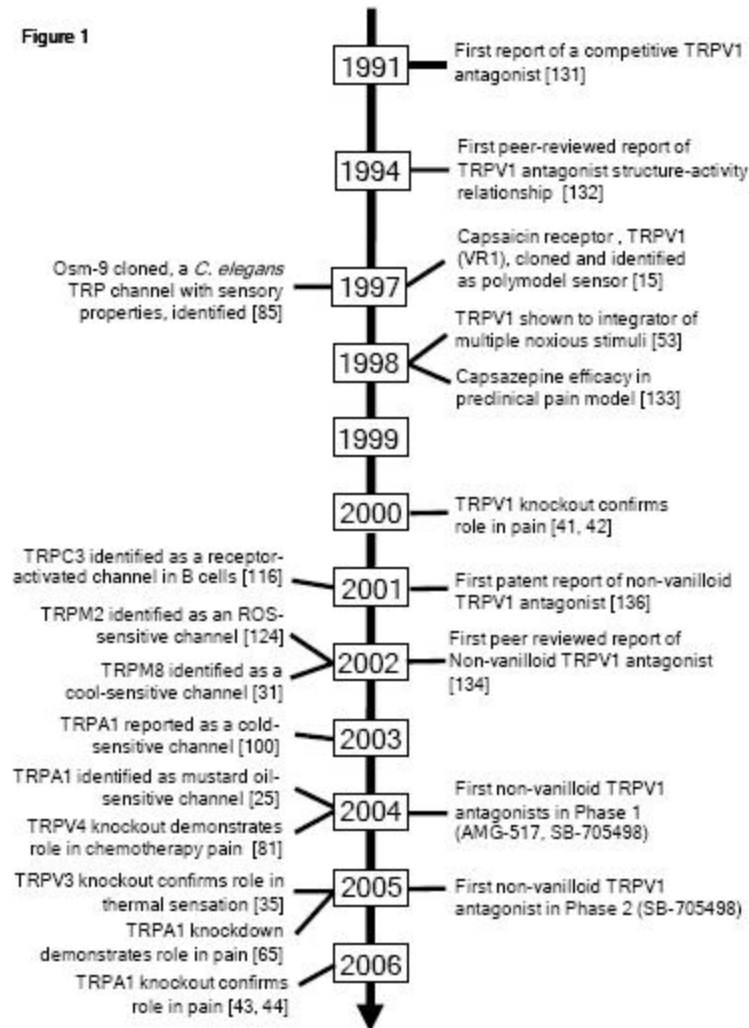
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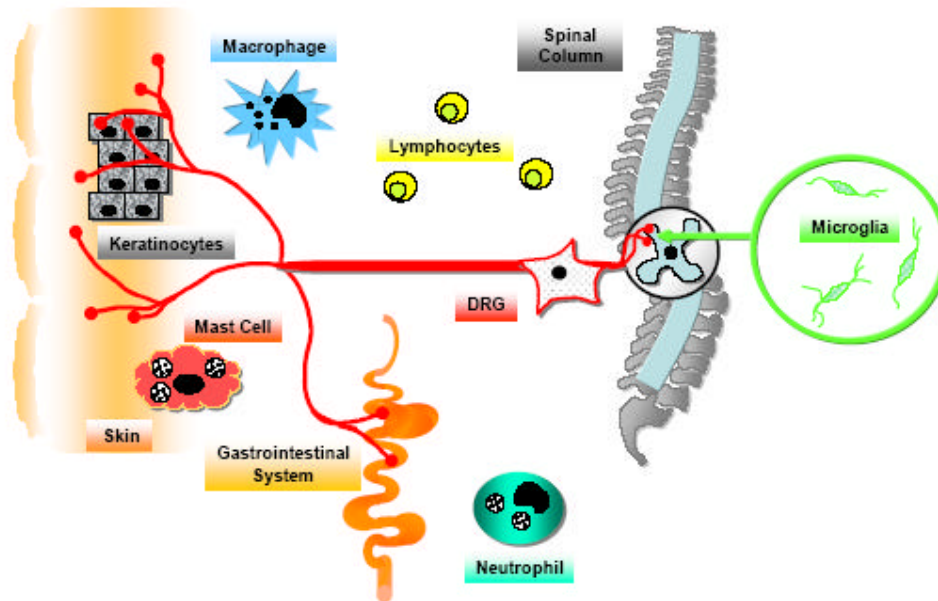
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Figure

Figure 1



Figure



ACCEPT